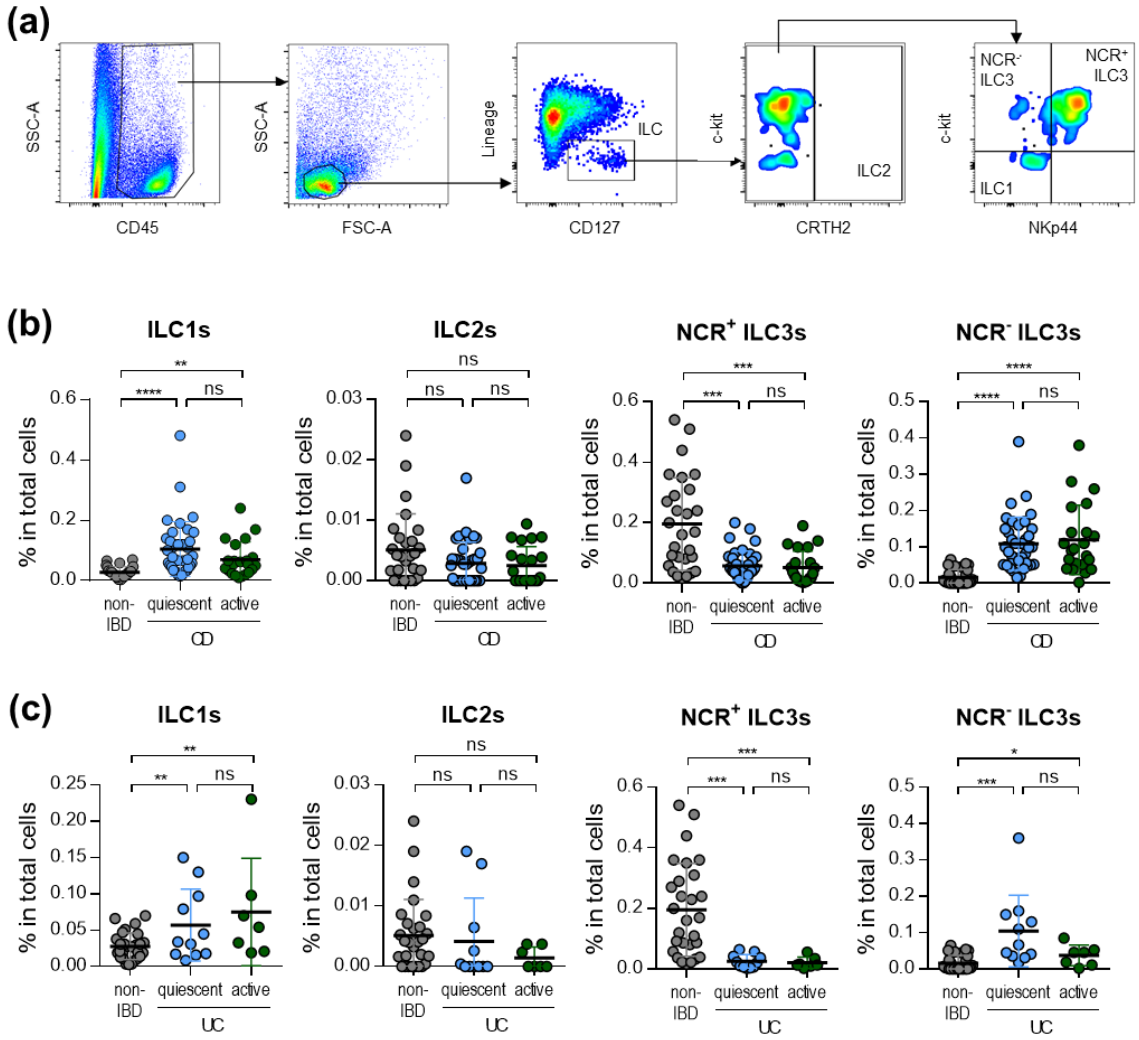
Supporting Information

Increased GM-CSF-producing NCR$^-$ ILC3s and neutrophils in the intestinal mucosa exacerbate inflammatory bowel disease

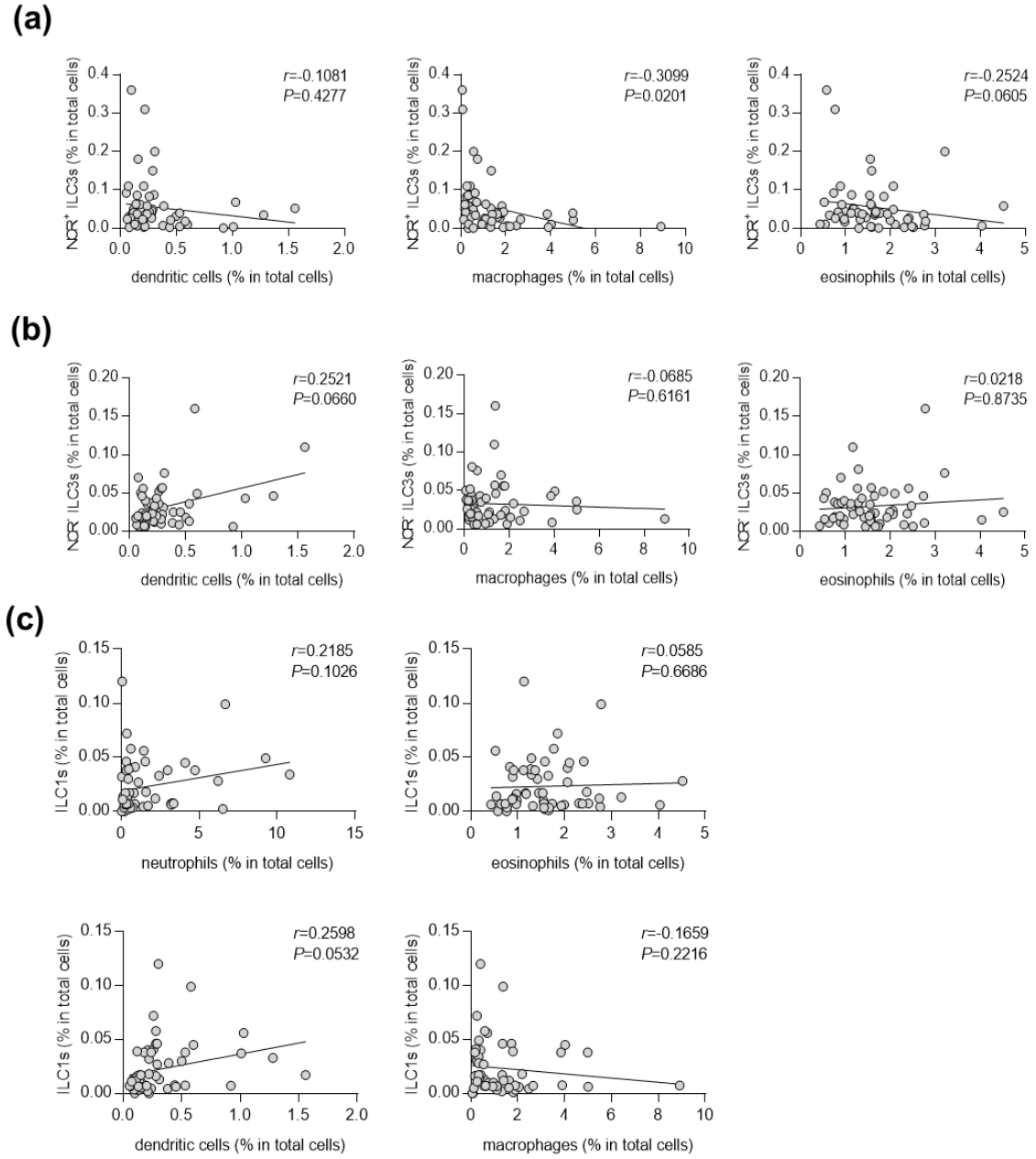
Yuna Chang, Ju Whi Kim, Siyoung Yang, Doo Hyun Chung, Jae Sung Ko, Jin Soo Moon, Hye Young Kim

Supplementary figure 1



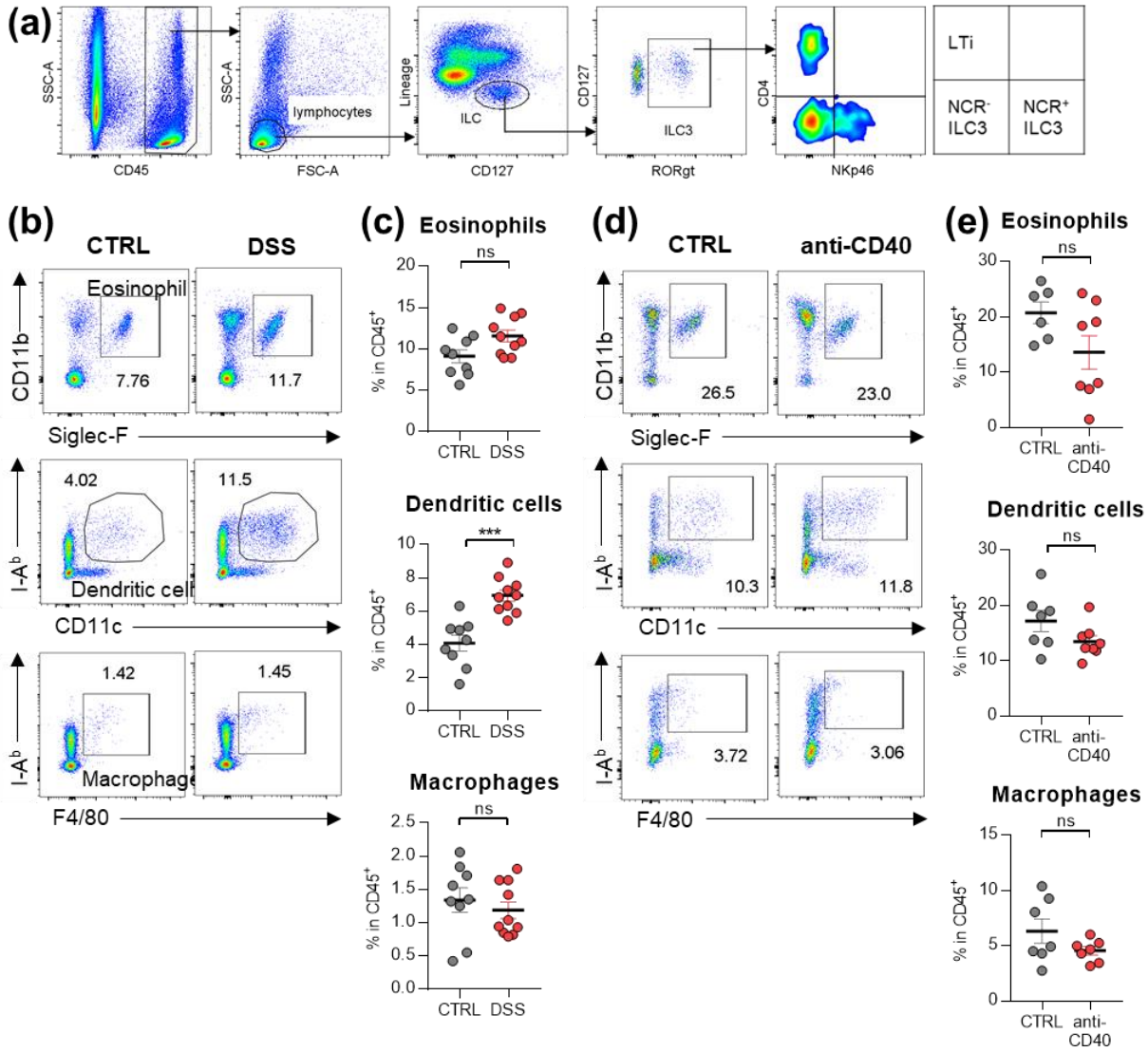
Supplementary figure 1. Gating strategy and the comparison of distribution of ILCs between Cronh's disease and ulcerative colitis. **(a)** Gating strategy for ILC1s ($CD45^+Lin^-CD127^+CRTH2^-c\text{-}kit^-NKp44^-$), ILC2s ($CD45^+Lin^-CD127^+CRTH2^+$), NCR^+ ILC3s ($CD45^+Lin^-CD127^+CRTH2^-c\text{-}kit^+NKp44^+$), and NCR^- ILC3s ($CD45^+Lin^-CD127^+CRTH2^-c\text{-}kit^+NKp44^-$). **(b)** The percentage of each subset of ILCs in colonoscopic biopsies of non-IBD subjects (n=30), cronh's disease with quiescent (n=38), and active status(n=22). **(c)** The percentage of each ILC subset in colonoscopic biopsies of non-IBD subjects (n=30), ulcerative colitis with quiescent (n=11), and active status (n=7). Data are presented as mean ± SD (One-Way ANOVA). *P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001, and ns, not significant (P > 0.05).

Supplementary figure 2



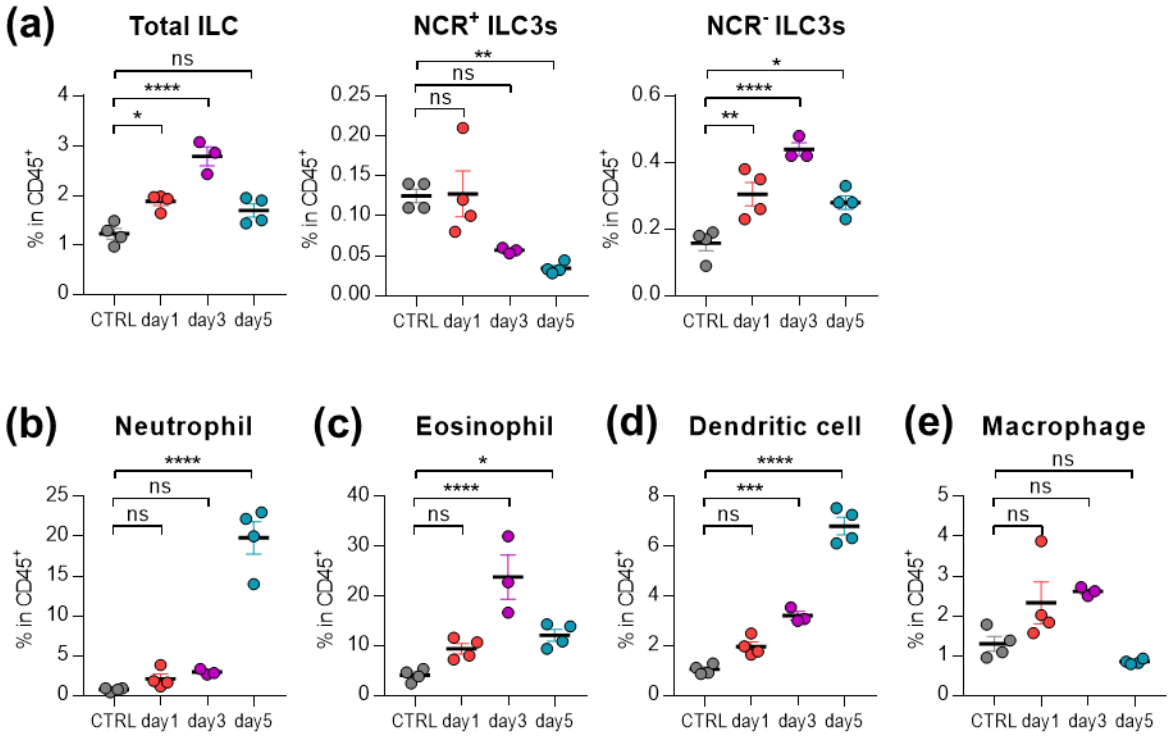
Supplementary figure 2. Correlation analysis between subsets of ILCs and myeloid cells. **(a)** Correlation of NCR$^+$ ILC3s with dendritic cells, macrophages, and eosinophils in non-IBD subjects and patients with IBD. **(b)** Correlation of NCR$^-$ ILC3s with dendritic cells, macrophages, and eosinophils in non-IBD subjects and patients with IBD. **(c)** Correlation of ILC1s with neutrophils, eosinophils, dendritic cells, and macrophages in non-IBD subjects and patients with IBD. The *r* and *P*-values are shown in the panel (Spearman's correlation test).

Supplementary figure 3



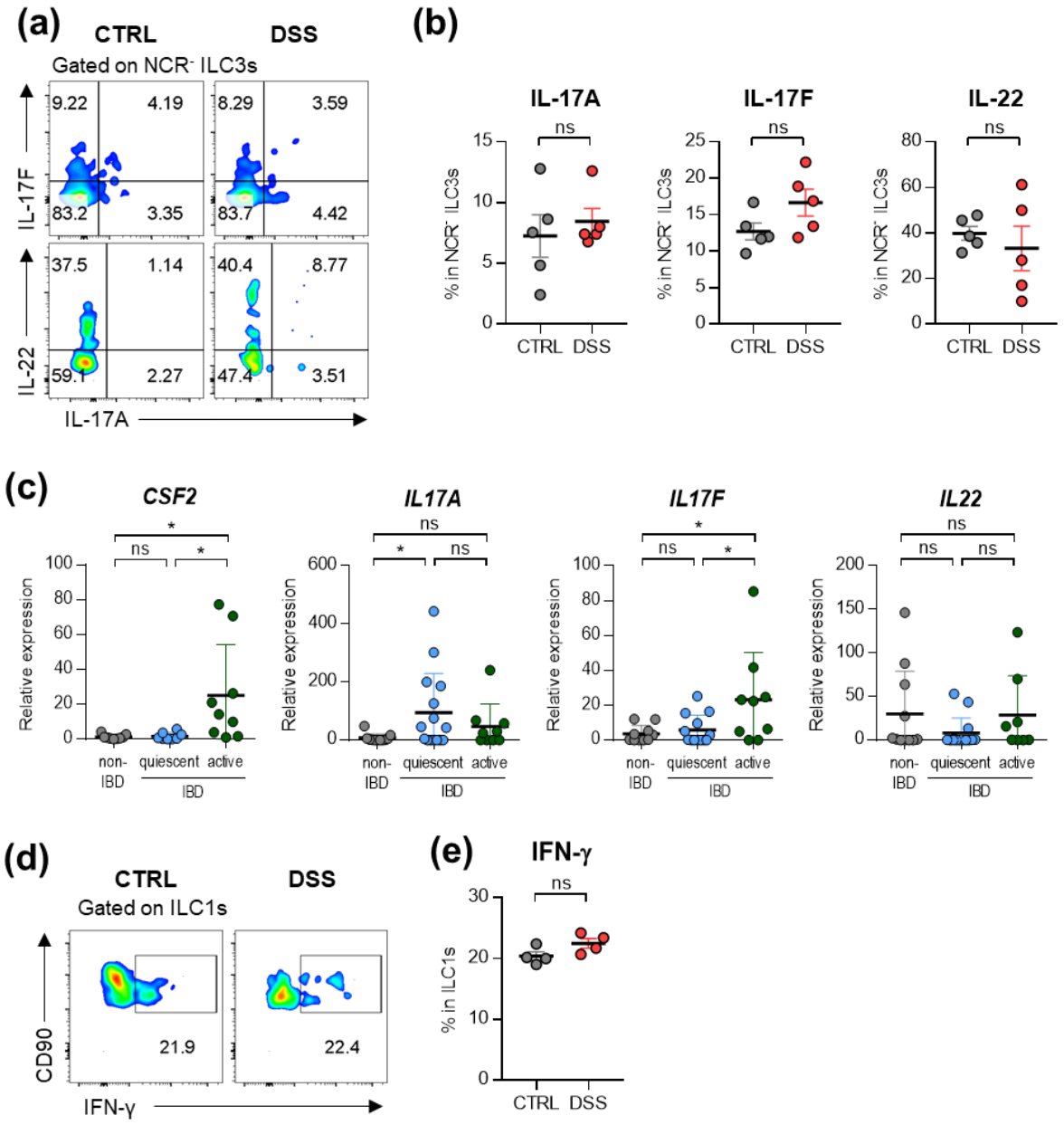
Supplementary figure 3. Altered immune cells in mouse models of colitis. **(a)** Gating strategy for LTis (CD45$^+$Lin$^-$CD127$^+$RORγt$^+$CD4$^+$), NCR$^+$ ILC3s (CD45$^+$Lin$^-$CD127$^+$ RORγt$^+$CD4$^-$ NKp46$^+$), and NCR$^-$ ILC3s (CD45$^+$Lin$^-$CD127$^+$RORγt$^+$CD4$^-$NKp46$^-$). **(b)** Representative flow cytometry dot plot of eosinophils (CD45$^+$CD11b$^+$Siglec-F$^+$), dendritic cells (CD45$^+$CD11c$^+$I-A^{b+}), and macrophages (CD45$^+$F4/80$^+$) in the colon at day 5 post induction with DSS. **(c)** The percentage of eosinophils, dendritic cells, and macrophages in the colon of control and DSS-induced mice. Data are presented as mean ± SEM (Student's *t*-test). Data were pooled from two independent experiments with n=5 per group. **(d)** Representative flow cytometry dot plot of eosinophils, dendritic cells, and macrophages in the colon at day 7 post induction with anti-CD40. **(e)** The percentage of eosinophils, dendritic cells, and macrophages in the colon of control and anti-CD40-induced mice. Results are presented as mean ± SEM (The student's *t*-test). Data are presented as mean ± SEM (Student's *t*-test). Data representative of two independent experiments with control (n=7) and anti-CD40 (n=8). **P* < 0.05, ***P* < 0.01, ****P* < 0.005, *****P* < 0.001, and ns, not significant (*P* > 0.05).

Supplementary figure 4



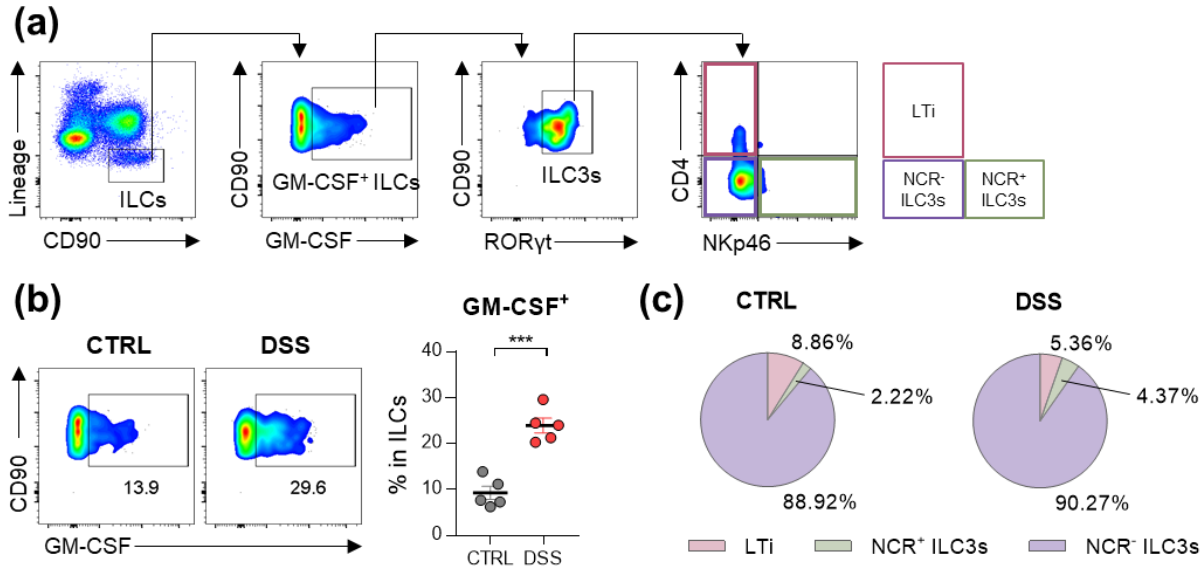
Supplementary figure 4. Immune cell kinetics in the colons of mice with DSS-induced colitis. **(a)** The kinetics of total ILCs, NCR$^+$ ILC3s, and NCR$^-$ ILC3s in the DSS-treated colon. Data are presented as mean ± SEM (One-Way ANOVA). **(b–e)** The kinetics of neutrophils (b), eosinophils (c), dendritic cells (d), and macrophages (e) in the DSS-treated colon. Data are presented as mean ± SEM (One-Way ANOVA). Data representative of two independent experiments with three or more mice per group. *P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001, and ns, not significant (P > 0.05).

Supplementary figure 5



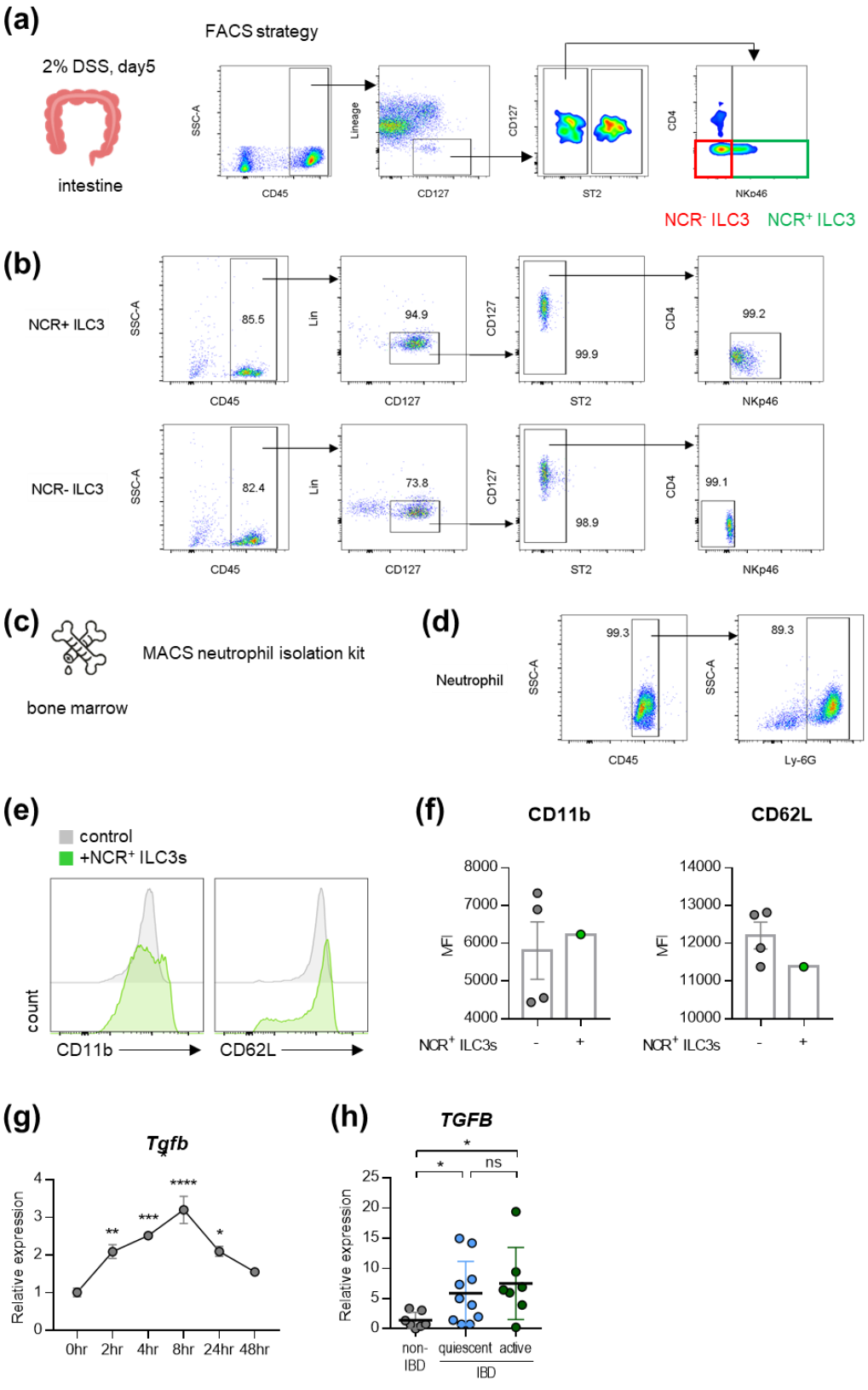
Supplementary figure 5. Other cytokines produced by ILCs in mouse models of colitis. **(a, b)** Representative flow cytometry dot plots (a) and the percentage (b) of IL-17A, IL-17F, and IL-22-producing NCR- ILC3s from the colon of mice with DSS-induced colitis. Data are presented as mean ± SEM (Student's *t*-test). Data representative of three independent experiments with n=5 per group. **(c)** Quantitative PCR analysis of *CSF2*, *IL17A*, *IL17F*, and *IL22* in human colonoscopic biopsies of non-IBD subjects (n=11) and IBD patients with quiescent (n=15) and active (n=9) status. Data are normalized to *RPLP0*. Data are presented as mean ± SD (One-Way ANOVA). **(d, e)** Representative flow cytometry dot plots (d) and the percentage (e) of IFN-γ-producing ILC1s from the colon of mice with DSS-induced colitis. Data are presented as mean ± SEM (Student's *t*-test). Data representative of two independent experiments with n=4 per group. *$P < 0.05$, ns, not significant ($P > 0.05$).

Supplementary figure 6



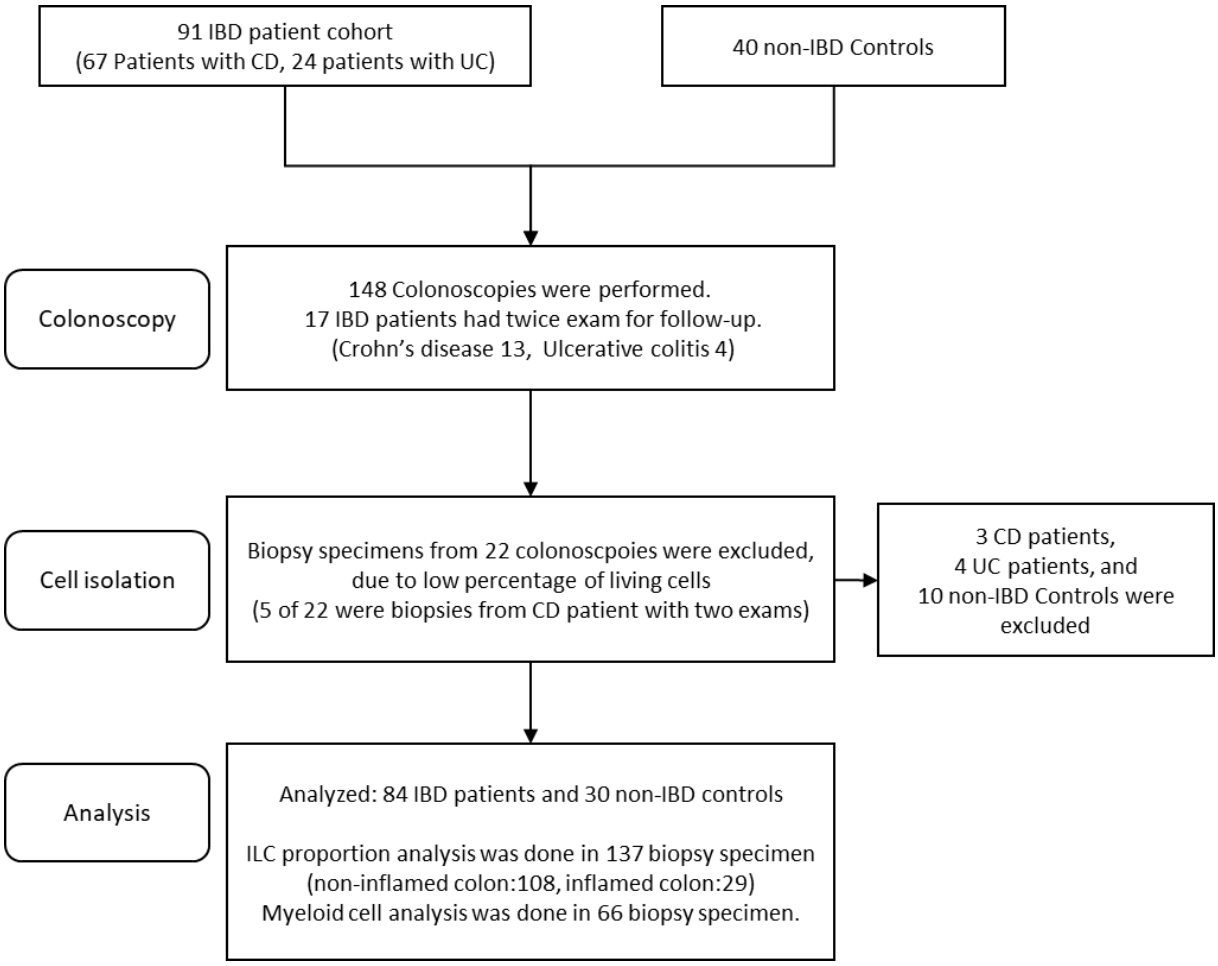
Supplementary figure 6. The proportion of ILC3 subsets in GM-CSF-producing ILCs. **(a)** Gating strategy for subsets of ILC3s in GM-CSF+ ILCs. **(b)** Representative flow cytometry plots and the percentage of GM-CSF+ ILC3s from the colon of mice with DSS-induced colitis. **(c)** Distribution of LTi, NCR+ ILC3s, and NCR- ILC3s in GM-CSF+ ILC3s from the colon of mice with DSS-induced colitis. Data are presented as mean ± SEM (Student's *t*-test). Data representative of three independent experiments with n=5 per group. ***$P < 0.005$.

Supplementary figure 7



Supplementary figure 7. NCR$^+$ ILC3s do not activate neutrophils. **(a)** Sorting strategy for NCR$^+$ ILC3s (CD45$^+$Lin$^-$CD127$^+$ST2$^-$CD4$^-$NKp46$^+$) and NCR$^-$ ILC3s (CD45$^+$Lin$^-$CD127$^+$ST2$^-$CD4$^-$NKp46$^-$). **(b)** The purity of sorted NCR$^+$ ILC3s and NCR$^-$ ILC3s is > 99%. **(c)** Neutrophils were isolated from bone marrow using magnetic-bead. **(d)** The purity of isolated neutrophils is > 89%. **(e, f)** Neutrophils and NCR$^+$ ILC3s were co-cultured for 24 hours. The representative histogram (e) and MFI (f) of CD11b and CD62L on neutrophils. Data representative of two independent experiments. Data are presented as mean ± SEM. **(g)** Neutrophils were isolated from bone marrow from naïve mice then stimulated with recombinant GM-CSF. The level of gene expression of *Tgfb* was measured at the indicated time point. Data are normalized to *Gapdh*. Data are presented as mean ± SEM (One-Way ANOVA). Data representative of three independent experiments with n=3 per group. **(h)** mRNA expression of *TGFB* in human colonoscopic biopsies of non-IBD subjects (n=7) and IBD patients with quiescent (n=10) and active (n=7) status. Data are normalized relative to *RPLP0*. Data are presented as mean ± SD (One-Way ANOVA). *P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001, and ns, not significant (P > 0.05).

Supplementary figure 8



Supplementary figure 8. Flow chart of this study.

Supplementary table 1. Demographics of the study population.

	IBD patient group		Control group n=30
	Crohn disease n=64	Ulcerative colitis n=20	
Sex			
Male	44	13	18
Female	20	7	12
Age, Mean(SD)	15.8 (2.5)	15.1 (2.8)	12.2 (3.8)
Clinical status *			
Active	32	12	N/A
Quiescent	40	12	N/A

*Six patients with Crohn's disease and four patients with ulcerative colitis had two consecutive colonoscopies.